Aquatic Toxicology, 9 (1986) 253–262
Elsevier

AQT 00224

THE USE OF BIOASSAYS TO ASSESS AQUATIC ARTHROPOD MORTALITY FROM PERMETHRIN DRIFT DEPOSITS

B.V. HELSON, P.D. KINGSBURY and P. DE GROOT

Forest Pest Management Institute, Canadian Forestry Service, Sault Sainte Marie, Ontario, Canada

(Received 15 April 1986; revised version received 9 September 1986; accepted 22 September 1986)

Three bioassay methods with 2 aquatic arthropods, *Gammarus pseudolimnaeus* (Crustacea: Amphipoda) and *Aedes aegypti* larvae (Insecta: Diptera), in artificial aquatic habitats were used to assess the acute lethal effects of permethrin due to downwind drift deposits from mistblower applications. In 3 separate tests, all methods produced similar results, namely, that single line sprays of permethrin at 35 g AI/ha resulted in mortality only within 30 m of the insecticide source. An application of 17.5 g AI/ha produced proportionately lower mortality. Mortality of amphipods was generally higher than that of mosquito larvae. The bioassay methods were very sensitive. Standards conducted concurrently with the tests gave LC_{50} values of 0.25–0.37 ppb for *G. pseudolimnaeus* and 0.69–1.85 ppb for *A. aegypti* larvae.

Key words: Permethrin; Mistblower; Spray drift; Bioassay; Toxicity; Aquatic arthropod; *Gammarus pseudolimnaeus; Aedes aegypti*

INTRODUCTION

The pyrethroid insecticide permethrin has excellent potential for forest insect pest management. It is highly toxic to many insect pests, particularly lepidopterous defoliators (Crisp, 1982; DeBoo, 1980), and has low mammalian and bird toxicity (Elliott et al., 1978). It is, however, very toxic to fish and some beneficial invertebrates, particularly aquatic insects and crustaceans (Anderson, 1982; Jolly et al., 1978; Muirhead-Thomson, 1978). In forestry applications of 70 g AI/ha or less, direct mortality of fish is unlikely to occur (Kingsbury, 1983). However, aerial applications directly over streams at dosages as low as 9 g/ha can result in severe disturbances to aquatic invertebrate communities and changes in the diets of resident fish populations (Kingsbury and Kreutzweiser, 1980). Such effects need to be avoided by the use of suitable buffer zones to eliminate the possibility of negative effects on fish and waterfowl.

In 1983, the Forest Pest Management Institute of the Canadian Forestry Service

Correspondence to: B.V. Helson, Forest Pest Management Institute, Canadian Forestry Service, P.O. Box 490, Sault Sainte Marie, Ontario, Canada P6A 5M7.

initiated a multidisciplinary study to establish the size of buffer zones that would be needed to protect aquatic habitats from significant invertebrate mortality due to downwind drift deposits of permethrin. This paper presents the results of one component of this study: the acute biological effects of deposits in aquatic systems downwind of mistblower applications. Mortality of 2 indicator aquatic invertebrates was measured by bioassays of water set out in jars, pans or pools. This approach was chosen for a number of reasons, the primary one being the capability this approach provided for testing responses to deposits at a variety of downwind distances and comparing these with standard bioassays at the same time. The use of 'artificial' static aquatic systems also allowed approaching 'worst case' conditions by maximizing surface-area-to-volume relationships, avoiding the dilution effects of flowing water systems and minimizing natural adsorptive surfaces (e.g., sediments and vegetation) that might reduce biologically active quantities of permethrin available to organisms. Possible sublethal effects of permethrin on growth, reproduction and population dynamics of invertebrates were not investigated in this study.

Similar approaches have been used by other researchers studying insecticide effects within and downwind of application sites. Womeldorf and Gillies (1968) used bioassays with mosquito larvae to determine swath widths and vegetative canopy penetration from low volume aerial sprays of chlorpyrifos. Schladweiler and Weigand (1983) also monitored the effects of permethrin and endrin drift from aerial applications for cutworm control with aquatic bioassays consisting of *Daphnia magna* in 250-ml beakers of water. In another study, *Drosophila melanogaster* adults were exposed to residues on Petri dishes to compare the effects of formulation on the drift of azinphosmethyl and malathion applied aerially (Argauer et al., 1968).

MATERIALS AND METHODS

Mistblower applications

In 1983, 2 trials were conducted in a flat, open field planted with white spruce (*Picea glauca*) with a mean height of approximately 0.75 m. In 1984, 1 trial was conducted in a similar but larger white spruce plantation with most trees less than 0.85 m in height.

A Solo Port 423 backpack mistblower was used to apply an aqueous emulsion of permethrin (Ambush 500EC) at a nominal dosage of 35 g AI/ha using a 10-m swath width. The applications were made along a spray line at right angles to the prevailing wind direction with the nozzle directed downwind about 1 m above ground level. Other application details and meteorological conditions during the sprays are presented in Table I. After each application, the residual volume in the mistblower was measured to calculate the actual dosage emitted.

TABLE I

Application details and meteorological conditions during permethrin mistblower applications.

	Trial 1	Trial 2	Trial 3
Date	13/09/83	27/09/83	18/07/84
Time	11:10	12:02	21:20
Nozzle type	Standard with 45° diffuser	Same as 1	ULV
Volume	25 l/ha	Same as 1	0.5 l/ha
Concentration AI	0.14%	Same as 1	3.5%
Emission rate	2 l/min	Same as 1	27 ml/min
Walking speed	1.3 m/s	Same as 1	0.9 m/s
Wind direction	NW	NW	NW
Mean wind speed (km/h)	9	8	14
Relative humidity (%)	80	53	64
Temperature (°C)	15	22	16

Bioassays

Two aquatic arthropods, *Gammarus pseudolimnaeus* and *Aedes aegypti* larvae, were used for the bioassays. The former were collected from nearby streams while the latter were reared in the laboratory using standardized techniques (Gerberg, 1970).

For both 1983 trials, 25 inflatable wading pools (about 0.8 m i.d.) were set up in the spray site along 5 lines, 20 m apart, starting 10 m downwind of the spray line. The pools were lined with 6-mil clear plastic and filled with 50 l of stream water. They were covered with plastic before treatment, uncovered just prior to the spray and left uncovered afterwards. Immediately after the spray, 20 3rd-instar mosquito larvae and 10 amphipods were placed in each pool in separate floating cages. A cage consisted of a white plastic container with the bottom and two 5 × 10-cm areas from opposite sides cut out and covered with fine mesh cloth screening.

Additional pools were set up 6 km from the spray site for use as untreated controls and treated standards. These standard pools were treated with a series of permethrin concentrations within 2 h of the sprays to obtain concentration–mortality relationships (Sun, 1963) for each trial. Two pools were treated with each quantity of permethrin to give 8 concentrations ranging from 0.01–16.0 ppb in Trial 1 and 0.05–8.0 ppb in Trial 2. Mortality of mosquito larvae was determined daily for 3 days and amphipod mortality was assessed periodically up to 9 days after treatment. Water samples were collected from each pool 0.5 h after the treatments for analysis by GLC for permethrin residues.

In the second 1983 trial, a set of three 500-ml glass Mason® jars filled with water was also placed adjacent to each of the 25 pool sites and at additional sites further downwind. One-half h after the spray the jars were collected and returned to the laboratory where 20 mosquito larvae were placed in each about 3 h after the spray.

Another group of jars was used as untreated controls and standards treated with permethrin to give concentrations ranging from 0.1–6.4 ppb in the jars. All jars were kept in a controlled environment chamber at 20°C and 14 h light:10 h dark. Mortality was assessed daily for 3 days.

In the 1984 trial, 10-l aluminum roasting pans, 50 × 30 × 8 cm deep, were used instead of wading pools. The pans were put in 8-cm deep holes in the ground. Five pairs of pans were placed at each of 5 distances downwind of the spray line. The pans were completely filled with river water and covered until the spray. One pan in each pair was exposed to 2 swaths while the other was exposed to only the 2nd swath. Additional pans were set up 1 km from the spray site and used as untreated controls and standards treated with permethrin to give 10 concentrations ranging from 0.4–25.6 ppb in the pans.

Beginning 15 min after the spray, two 500-ml Mason jars were filled with water from each pan after stirring. The jars were returned to the laboratory where 20 4th-instar larvae were placed in each about 4 h after the spray. A set of jars from each pan was kept outdoors while the other set was kept in an environmental chamber at 20°C. Mortality was checked daily for 4 days. Results from the 2 sets of jars were similar and have been combined for analysis.

Data analysis

Probit analysis was performed on the concentration–mortality data from the standards using a computer program based on Finney (1971). Unless otherwise stated, all percent mortalities have been adjusted for natural mortality in the untreated controls by Abbott's formula (Abbott, 1925).

RESULTS

Trial 1 (1983)

In this trial, the actual dosage of permethrin emitted from the mistblower was 33.5 g AI/ha. Mortality of *G. pseudolimnaeus* was rapid and high after 48 h in all pools 10 m from the spray line and substantial mortality also occurred in 1 pool at 30 m (Table II). In the other pools at 30 m and further downwind, mortality was low after 48 h, and no major increase in death occurred up to 6 days after treatment. Permethrin was very toxic to amphipods in the standard pools. Probit analysis of the data provided 48 h LC_{50} and LC_{95} values with confidence limits of 0.25 ppb (0.10, 0.42) and 4.41 ppb (2.35, 15.32), respectively. When compared to the toxicity of permethrin in the standard pools, the correspondence between measured permethrin residues and the amphipod mortality observed in pools exposed to the mistblower application was generally good. At 10 m, the mean permethrin residue in the 5 pools was 0.90 ppb (range 0.19–1.96 ppb). This exceeded the LC_{50} value

TABLE II

Mortality of *G. pseudolimnaeus* and *A. aegypti* larvae after 48 h in wading pools exposed to permethrin drift deposits.

Distance downwind (m)	Mean percent of mortality (n = 5)		
	Trial 1	Trial 2	
	Gammarus	*Gammarus*	*Aedes*
10	95 (76–100)[a]	85 (37–100)	76 (37–100)
30	12 (0–52)	18 (5–37)	6 (0–14)
50	5 (0–23)	0	2 (0–10)
70	6 (0–15)	2 (0–5)	0
90	1 (0–3)	1 (0–5)	0
Untreated	18[b] (10–30)	5[c] (0–10)	13[c] (0–32)

[a] Range; [b] $n=4$; [c] $n=6$.

from the standard pools and correspondingly high mortality was observed at this distance (Table II). At 30 m, the mean residue of between 0.010–0.014 ppb (range ND[1]–0.03 ppb) was well below the LC_{50} value which again corresponded with the relatively low mortality observed at this distance. Mosquito larvae were also placed in the pools but high control mortality and erratic mortality in treated pools occurred, probably because of the low minimum water temperatures (4°C) experienced during the trial.

Trial 2 (1983)

In the second 1983 trial, the actual dosage of permethrin was 33.1 g AI/ha. Mortality of both *G. pseudolimnaeus* and *A. aegypti* after 48 h was high in most pools at 10 m with some mortality also evident in the pools at 30 m (Table II). The mortality of amphipods increased progressively to 100% at 10 m and 64% at 30 m by 9 days after treatment. At distances beyond 30 m, mortality of both arthropods remained low or did not occur at any time after treatment.

Permethrin was more toxic to *G. pseudolimnaeus* than to *A. aegypti* larvae in the standard pools. LC_{50} and LC_{95} values for amphipods at 48 h were 0.37 ppb (0.32, 0.43) and 0.61 ppb (0.51, 0.76), respectively, whereas these values were 0.69 ppb (0.59, 0.79) and 1.14 ppb (0.97, 1.52) for mosquito larvae. The slope of the probit line for amphipods in this trial (7.53) was much steeper than in Trial 1 (1.33). This may have been due to the higher minimum water temperatures in Trial 2 (13°C) than in Trial 1 (4°C) during the 48-h period after treatment.

Mean permethrin residues were 1.95 ppb (range 0.49–4.07 ppb) at 10 m; 0.15 ppb (range 0.04–0.28 ppb) at 30 m and between 0.038 and 0.042 ppb (range ND–0.11 ppb) at 50 m. Compared to the LC_{50} values, these concentrations would be expected

[1]ND = nondetectable, detection limit = 0.01 ppb.

TABLE III

Mortality of *A. aegypti* larvae after 72 h in jars exposed to permethrin drift deposits in Trial 2.

Distance downwind (m)	Percent of mortality[a] at line				
	1	2	3	4	5
10	100***[b]	100***	47 ± 10***	43 ± 37***	100***
30	10 ± 5**	5 ± 9 NS	0	32 ± 7***	5 ± 5 NS
50	2 ± 3 NS	0	0	7 ± 8 NS	3 ± 6 NS
70	0	0	0	2 ± 3 NS	3 ± 3 NS
90	2 ± 3 NS	0	0	5 ± 0+	0
110	5 ± 5 NS	2 ± 3 NS	5 ± 5 NS	2 ± 3 NS	5 ± 5 NS
130	2 ± 3 NS	0	3 ± 3 NS	0	3 ± 3 NS
150	0	3 ± 3 NS	0	12 ± 17 NS	6 ± 10 NS

Mean percent of mortality in untreated jars 1.5 ± 2.4 ($n=10$).

[a] Mean of three jars at each site ± SD not adjusted for control mortality.

[b] Student's *t*-test after arc sin transformation.

*** Significantly different than the untreated mortality at $P=0.001$.

** Significantly different than the untreated mortality at $P=0.01$.

* Significantly different than the untreated mortality at $P=0.05$.

NS = not significantly different than the untreated mortality at $P = 0.05$.

to give high mortality at 10 m, relatively low mortality at 30 m and very little if any mortality at 50 m. These correspond with the observed mortalities at these distances (Table II).

The mortality of *A. aegypti* larvae in glass Mason jars at all 10-m sites and at 30 m on lines 1 and 4 was significantly higher than in untreated jars (Table III). Little or no mortality occurred at the other sites at 30 m and further downwind. LC_{50} and LC_{95} values at 72 h in standard jars for this trial were 1.52 ppb and 5.76 ppb, respectively. Because the regression of probit mortality with log concentration was not significant in this test, no confidence limits were obtainable.

TABLE IV

Mortality of *A. aegypti* larvae after 84 h in jars of water from aluminum pans exposed to permethrin drift deposits in Trial 3.

Distance downwind (m)	Mean percent of mortality ($n=5$)	
	One swath	Two swaths
10	59 (31–100)[a]	72 (32–100)
30	2 (0–6)	3 (0–6)
50	2 (0–4)	2 (0–3)
100	1 (0–1)	2 (0–4)
200	1 (0–1)	1 (0–3)
Untreated	2 (0–5)	2 (0–5)

[a]Range.

Trial 3 (1984)

In the 1984 mistblower trial, the actual dosage of permethrin was 35.0 g AI/ha. Mortality of *A. aegypti* larvae consistently occurred in water samples from the aluminum pans at 10 m exposed to either 1 (17.5 g AI/ha) or 2 swaths (35 g AI/ha) (Table IV). Overall mortality was proportionally lower with 1 swath than 2. Very little mortality occurred further downwind with either exposure. The toxicity of permethrin in water samples from standard aluminum pans in this trial was comparable with the standard Mason jars in the second 1983 trial. LC_{50} and LC_{95} values at 84 h were 1.70 ppb (0.96, 2.65) and 7.87 ppb (4.34, 44.04), respectively.

DISCUSSION

The similarities in lethal effects of downwind deposits of permethrin from a single spray line on the indicator aquatic arthropods in these different trials are illustrated in Fig. 1. In all trials, relatively high mortality occurred at 10 m, the downwind edge of the swath width of the mistblower. Mortality of *G. pseudolimnaeus* was higher than that of *A. aegypti* larvae, which is consistent with the relative toxicity of permethrin to these arthropods. As expected, the lowest mortality occurred with *A. aegypti* larvae at 17.5 g AI/ha. Mosquito larvae exposed to 35 g AI/ha using 3 different bioassay methods in 2 trials exhibited very similar mortalities ranging from

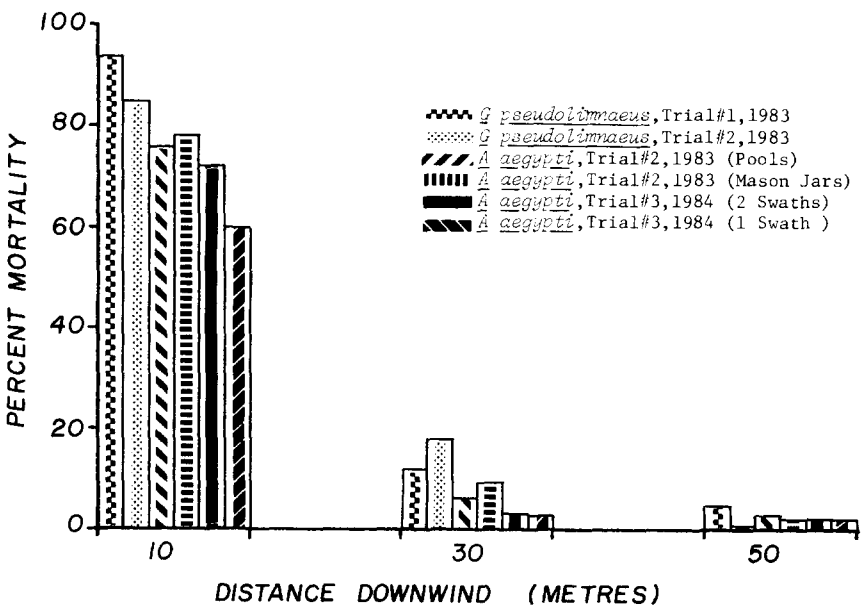

Fig. 1. Mean percent mortality of aquatic arthropods at each distance downwind of permethrin mistblower applications.

72–78%. Similar trends were evident at 30 m although mortality was much less and more variable than at 10 m. At 50 m, mean mortality was less than 5% in any bioassay, indicating very little if any acute lethal impact due to permethrin drift at this distance. Permethrin residue levels in water samples from wading pools in the first 2 trials supported these trends in bioassay results.

The bioassays were very sensitive. The toxicity of permethrin to *A. aegypti* larvae in the standard pools, jars and pans in these trials was comparable to published values for this (Herald et al., 1980) and other mosquito species (Mulla et al., 1980; Rettich, 1979) in laboratory toxicity tests. Although we are not aware of any publications reporting the toxicity of permethrin to a *Gammarus* species under static water conditions, Muirhead-Thomson (1978) obtained a 24-h LC_{90-95} of 1 μg/l with *Gammarus pulex* in a continuous water flow system after 1 h exposure. The toxicity of permethrin to *G. pseudolimnaeus* in standard pools was also very similar to that reported for *Daphnia magna* in laboratory tests (Stratton and Corke, 1981). The present bioassays were designed to maximize the toxicity of the permethrin deposits to these indicator arthropods and the observed responses of the test organisms indicate that this was accomplished. In many natural water bodies, the lethal effects of comparable downwind deposits of permethrin would probably be less due to absorption of the insecticide on organic matter present in the water. Rettich (1980) found that the concentrations of permethrin required to control *Aedes vexans* larvae under field conditions were higher than anticipated from laboratory tests, presumably for this reason.

Although the different bioassay methods with *A. aegypti* larvae yielded similar results, the Mason jar and aluminum pan techniques did have some advantages over the wading pool approach. The former were less expensive, much simpler and faster to assemble and dismantle, and eliminated the use of plastic surfaces, which can absorb insecticides. Furthermore, the larvae can be held under specific and controlled environmental conditions if desired. Control mortality was also much less than in the pools.

The mortality caused by downwind deposits of permethrin in this study represents the situation for a mistblower treatment along a single spray line. The potential mortality resulting from permethrin deposits at various downwind distances when the insecticide is emitted along a number of lines separated by the swath width of the mistblower can now theoretically be determined through appropriate mathematical modelling based on these biological data as well as actual permethrin deposits at different downwind distances. These deposits were measured as a separate component of this study and the results of this analysis will be reported elsewhere (Payne et al., 1986). The only similar study designed to assess the biological effects of spray drift deposits of a pyrethroid in freshwater is that by Crossland et al. (1982) who assessed the effects of operational mistblower treatments of cypermethrin at 30 to 45 g AI/ha in streams adjacent to treated vineyards in France. Under such conditions, the average deposit on the surface of the streams was 5–10% of the deposit in the

vineyards and at worst this contamination caused only minor and temporary disturbances to the invertebrate fauna. It was not stated if the streams were downwind of the vineyards at the time of the treatment. Cypermethrin is more toxic than permethrin to aquatic invertebrates in studies where direct comparisons have been made (e.g., McLeese et al., 1980; and Mulla et al., 1978).

The use of bioassays with indicator invertebrates in artificial aquatic habitats to assess the impact of insecticide drift offers a number of advantages over other approaches. They are relatively simple to perform and provide the opportunity for replication and testing different variables simultaneously. They provide direct data on mortality or other toxic effects if desired and eliminate several assumptions required when translating quantities of deposits on collecting surfaces to toxicity in water. They are particularly valuable when used in conjunction with deposit collections for assessing the biological significance of the measured deposits. Finally, bioassays eliminate the need to contaminate natural bodies of water and avoid the tedious sampling required for such systems.

ACKNOWLEDGEMENTS

We gratefully acknowledge the time and effort spent by Dr. K.M.S. Sundaram and his staff in analyzing the water samples for permethrin residues; and Dr. N. Payne for obtaining the site and providing the weather measurements in 1984. We thank Dr. G.B. Craig, Jr., University of Notre Dame, for providing *A. aegypti* eggs; and Chipman Inc. for supplying the Ambush 500EC for this study. The technical assistance of John McFarlane, Dave Kreutzweiser, Andrij Obarymskyj, Dave Comba, Steve Holmes, and seasonal staff is acknowledged with thanks.

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